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(57) Abstract

A system for simultaneously conducting multiple ligand assays on a sample potentially containing target analytes uses as a detector a waveguide having thereon a plurality of probes of known recognition to the target analytes. The probes are in discrete areas on the waveguide. A sample containing target analyte is treated with a light-responsive compound such that it binds to the target analyte to form a conjugate and the conjugate is applied to the probes on the waveguide. A laser light is passed into the waveguide so that evanescent waves radiate from the waveguide. Where conjugate has attached to probe there is emission of light different from that emitted by a probe without conjugate attached thereto.

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SYSTEM FOR SIMULTANEOUSLY CONDUCTING MULTIPLE LIGAND BINDING ASSAYS

BACKGROUND

This invention is directed to analysis of a biological fluid sample.

Health care costs consume a significant percentage of the gross national product. A substantial portion of the cost of health care is attributed to laboratory testing, which can be labor-intensive. Such laboratory testing can involve testing for multiple analytes in a patient's blood, urine, or spinal fluid.

Time is of the essence in laboratory testing. In certain instances, the successful treatment of a patient requires quick results from laboratory tests.

Sensitivity is also important in laboratory tests. Oftentimes, a substance of interest is present in minute quantities. In many situations, accurate diagnosis of a patient's condition requires the ability to detect these minute quantities. Existing testing systems are usually inadequate in that they suffer from at least one of the following disadvantages: high cost, slow results, or low sensitivity.

Accordingly, there is a need for a system for economically analyzing biological fluids which (1)Êis low cost; (2) provides quick results; and (3) can detect small quantities of analytes.

SUMMARY

The present invention is directed to a system that satisfies these needs. In particular, this system inexpensively and quickly detects analytes at low concentrations. The system can simultaneously test for multiple analytes or can test multiple samples for a single analyte. The system has the added advantage that it can be automated with non-complex machinery.

The system includes a method for detecting a target analyte in a sample utilizing a unique detector, which comprises a waveguide having thereon a plurality of discrete probes. Each probe includes a specific binding partner for a selected analyte, and at least one of the probes is a responsive probe that includes a specific binding partner for the target analyte. Preferably the specific binding partner is covalently bonded to the waveguide. The sample is applied to the detector such that the target analyte binds to its specific binding partner. Laser light is passed into the detector so that evanescent light radiates from the waveguide and impinges on the probes. Any light emitted from a probe with target analyte bound thereto is different from the light, if any, emitted by the same probe without target analyte bound thereto. Emission of light from the probes is detected, and from such detection, the presence or absence of the target analyte in the sample can be determined.

This differential in light emission can be effected by attaching a light-emitting compound, such as a fluorophore, to the target analyte, either before or after it binds to its specific binding partner. The light-emitting compound emits light when the laser light is passed into the detector. For example, the fluorophore can be bound to a second specific binding partner for the target analyte, wherein a sandwich is formed on the detector in the sandwich comprising the fluorophore, the second specific binding partner, the target analyte, and the specific binding partner.

Alternatively, the probes can include a light-emitting compound, where the presence of the target analyte binding to the probe affects the light emitted by the light-emitting compound.

In another version of the invention, instead of using a light-emitting compound, a light-modulating compound, such as a fluorophore quencher or an enhancer, can be attached to the target analyte, where the probe has pre-attached to it a light-emitting compound whose

light-emitting properties are affected by the light-modulating compound.

The system of the present invention can be used for screening multiple samples for the selected target analyte. This can be effected by using different light-emitting compounds for attachment to the target analyte in different samples, wherein the different light-emitting compounds emit light having a detectable difference when exposed to the evanescent light.

This system also can be used for detecting multiple target analytes in a single sample. This is effected by using a detector having a plurality of different discrete probes, wherein at least some of the probes are capable of binding to target analytes in the sample. The sample is applied to the detector such the target analytes bind to a corresponding specific binding partner. Laser light is passed into the detector so that evanescent light radiates from the waveguide and impinges on the probe, wherein light, if any, emitted from a probe with target analyte bound thereto is different from the light, if any, emitted by the same probe without target analyte bound thereto. By detecting the emission of light from the probes, it is possible to determine which of the target analytes are present in the sample.

The present invention can be used both for qualitative analysis, and quantitative analysis. For quantitative analysis, a known quantity of an analog of the target analyte is applied to the detector for a competitive assay.

The waveguide can have a water permeable overlayer with an index of refraction less than the index of refraction of the waveguide, such as a sol gel. The overlayer can improve the containment of the laser beam within the substrate and thereby reduce background noise and interference.

In another version of the invention, the refractive index of the overlayer is substantially equal to or greater than the refractive index of the substrate. In this version, the light-emitting compound is excited

by epi-illumination by a laser light source positioned perpendicular to the plane of the substrate. At each discrete area, emission of light of a wavelength different from that of the incident laser is detected.

A preferred detector comprises a waveguide having first and second opposing surfaces. The index of refraction of the waveguide is greater than its surrounding medium. Accordingly, when a laser light is passed into the waveguide at an angle of incidence greater than a critical angle, evanescent waves radiate from the first and second surfaces. There are a plurality of probes of known recognition to selected target analytes in discrete areas on at least one of the surfaces of the waveguide. The waveguide can be substantially planar with the probes in a two-dimensional array, with a fluorophore included with the probes. For example, the probes can be a plurality of discrete spots printed onto the surface of the waveguide.

The present invention also includes an apparatus effective for transmitting the light from a laser light source, which typically emits an approximately pencil-shaped beam, to a beam that is suitable for introduction into the end of a waveguide.

This system has the advantage of involving only a small number of simple steps which in turn facilitates automation by non-complex machinery. For example, in one version of the invention, there are only three manipulative steps; *i.e.*, (1) applying sample to a waveguide wherein a light-responsive compound has been included with the probes on the waveguide, (2)Êpassing a laser light into the waveguide, and (3) detecting emitted light.

The assay is sensitive enough to detect a target analyte at low concentrations. It has been demonstrated down to 10_{-13} molar. This invention provides a considerable time and cost advantage over prior art methods. As such, the invention is economical, time efficient and sensitive.

DRAWINGS

These and other features, aspects and advantages of the present invention will become better understood from the following written description of the invention, the appended claims and accompanying drawings where:

Fig. 1 is a perspective view of a waveguide according to the present invention with a two-dimensional array of probes in the shape of dots on a surface of the waveguide;

Fig. 2 is a perspective view of a second waveguide according to the present invention with spaced apart probe strips on the waveguide, with a sol gel overlayer;

Fig. 3 is a perspective view of a third waveguide according to the present invention with dot-shaped probes on both surfaces of the waveguide;

Figs. 4A and 4B are cross-sectional views of the waveguides of Fig. 1 and 2, respectively, showing light entering the waveguide at an angle greater than a critical angle;

Fig. 5 is a schematic diagram of an apparatus for conducting analysis according to the present invention;

Fig. 6 is a perspective view showing the cylindrical optics of the apparatus of Fig. 5 and the light path through the cylindrical optics;

Fig. 7 is calibration curve for a competitive immunoassay to quantitatively analyze Digoxin in a sample analyzed according to the present invention; and

Fig. 8 presents a CCD generated image for a qualitative hybridization analysis for DNA oligonucleotides in a sample analyzed according to the present invention.

DESCRIPTION

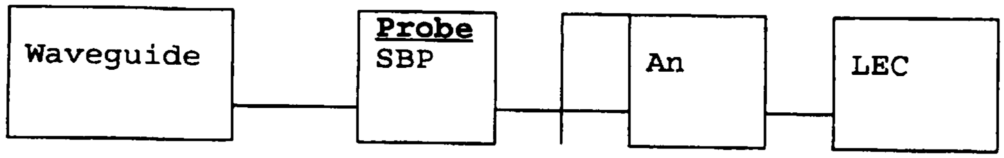
The present invention is directed to a system for simultaneously conducting multiple ligand binding assays on a sample possibly containing target analytes, wherein a complex between a specific binding partner at a probe and target analyte is detected using evanescent waves and light-responsive compound or compounds.

Referring to Fig.Ê1, a plurality of different probes **20** (designated by letters aa to xx) having specific binding partners are applied in discrete areas **22** onto the top **23** and bottom surfaces **25** of a planar waveguide **24**. The waveguide **24** has an index of refraction greater than its surrounding medium, which is usually air. Each probe **20** includes a specific binding partner of known recognition to a selected target analyte; *e.g.*, a monoclonal antibody, DNA oligonucleotide or enzyme cofactor. More than one probe can be bound to a particular analyte, i.e., there can be multiple probes responsive to an analyte such as Digoxin for improved sensitivity. In fact, all of the probes **20** can be responsive to a single analyte.

A sample is applied to the waveguide **24** such that target analytes in the sample bind to the specific binding partner at a responsive probe. Referring to Fig. 4A, laser light **42** is passed into the waveguide **24** at an angle greater than a critical angle (defined below) so that there is total internal reflection **28** of the laser light and evanescent waves **30** radiate from the surface of the waveguide.

There are multiple variations of this system. In a first variation a light-emitting compound that is luminescent is pre-bonded to target analyte molecules (*e.g.*, DNA/RNA, proteins, polysaccharides and the like) in the sample prior to applying the sample to the waveguide. At those discrete areas where a target analyte forms a complex with a responsive probe, light is emitted. This first version can be schematically represented as:

(1)



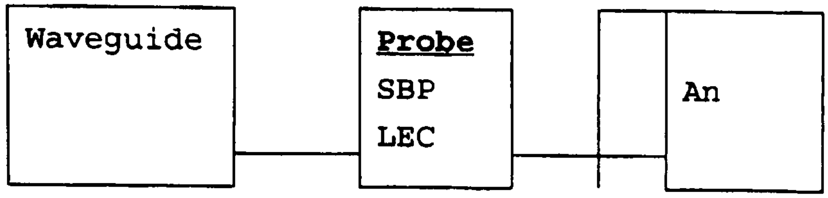
Wherein "SBP" stands for "specific binding partner; "An" stands for "target analyte" and "LEC" stands for "light emitting compound."

Multiple (at least two) light-emitting compounds which emit light at different wavelengths and which bond to different target analytes can be used. This allows simultaneous detection of different target analytes or samples with a single waveguide in a single test.

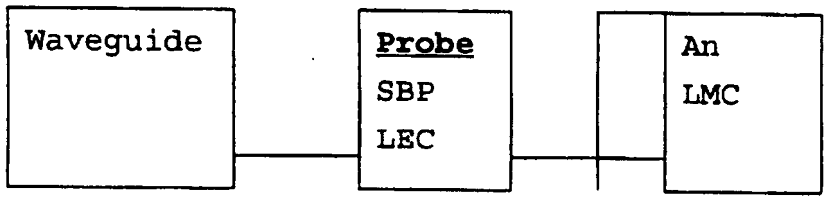
Alternatively, multiple light-emitting compounds which emit light at different wavelengths but bond to the same target analyte are used to simultaneously analyze multiple samples for the target analyte. For example, if the target analyte is present in the first sample, a first LEC emits, and if it is present in the second sample, a second LEC emits light at a different wavelength.

In a second variation, the light-emitting compound is included with the specific binding partner at each probe at each of the discrete areas, rather than being initially bonded to the target analyte. At those discrete areas where target analyte in•sample has bonded to a specific binding partner at a probe, the emission of light is either enhanced or quenched.

This second version can be schematically represented as:



2(a)



2(b)

where "LMC" stands for "light modulating compound"; *i.e.*, a light enhancer, quencher or wavelength shifter. In this second variation, the waveguide is provided with a probe that includes the LEC and a specific binding partner for the target analyte. In the detection step, the analyte alone (2(a)) or the analyte with a LMC (2(b)) is attached to the SBP.

Multiple different light modulating compounds having different modulating effects can be used to simultaneously detect multiple target analytes in the sample. LMCs that cooperate with one another are selected; *i.e.*, the effect of one does hinder or cancel out the effect of another. This can improve the detection and discrimination between different target analytes as well as to further facilitate the simultaneous assay of different classes of target analytes. The different LMCs bond to different target analytes or samples and modulate the LRC differently.

Alternatively, different LMCs with different modulating effects can be used for bonding to the same target analyte but in different samples. This variation provides for the simultaneous assay of different samples for the target analyte.

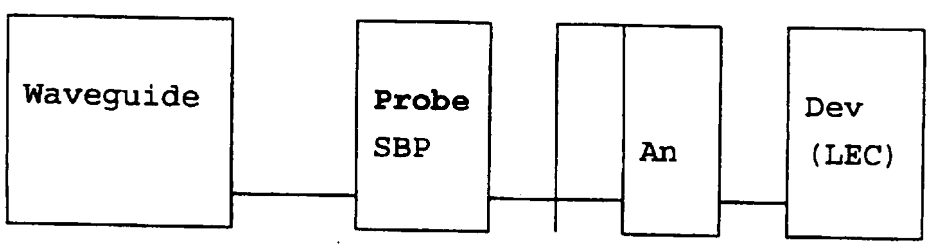
In a third variation of the invention, after applying the sample to the waveguide, a developer is applied to the waveguide. The developer comprises a

light-emitting compound, which attaches to the target analyte bonded to a specific binding partner to form a conjugate or sandwich.

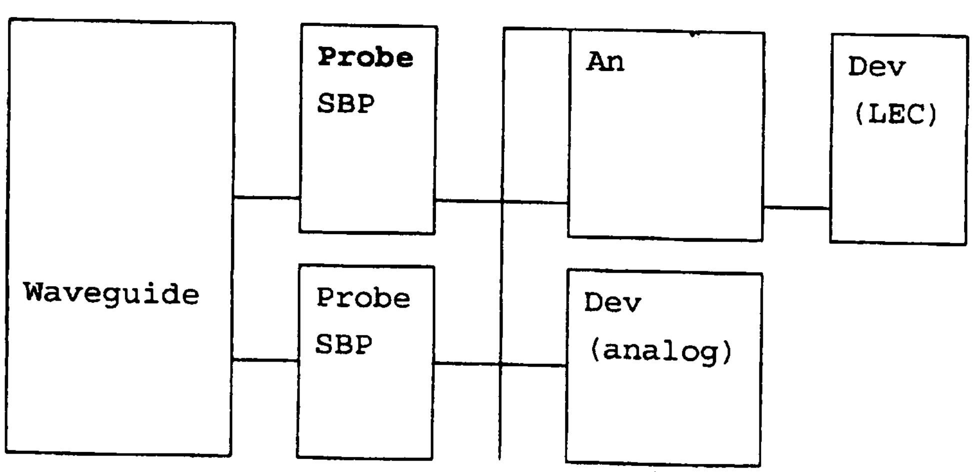
The system can be used for quantitative competitive assay. For example, the developer can include a known quantity of an analog of the target that competes with target analyte to bond to the probe. Thus, the target analyte and the analog bind to the probe in a proportion based on their respective concentrations. The waveguide is treated with a light-emitting compound which is specific for attaching to the target analyte but does not bond to the analog. The greater the amount of target analyte which bonds to the probe, the more light-emitting compound is present at the probe and the stronger the light emission signal.

Alternatively, the light-emitting compound can be specific for attaching to the analog rather than the target analyte. Consequently, the greater the concentration of target analyte compared to the analog concentration, the lower the signal strength. A calibration curve is used to convert signal strength to concentration of target analyte.

This third variation can be schematically represented as:



3 (a)



3 (b)

wherein "Dev" stands for "Developer". Schematic 3(a) depicts a qualitative binding assay, and schematic 3(b) depicts a quantitative competition assay.

Waveguide -- The waveguide **24** is a substantially optically transparent, planar sheet of material. It has a sufficiently high refractive index relative to its surrounding medium that it contains incident light that impinges upon it and transmits the light when the angle of incident light is greater than the critical angle. The critical angle **26** is the arcsine of the quotient of the refractive index of the surrounding medium divided by the refractive index of the waveguide **24**. Under these conditions an electromagnetic field radiates beyond the surface of the waveguide. This field, which is known as an evanescent wave, penetrates into the surrounding medium and has the ability to excite a light-emitting compound (*e.g.*, a fluorophore) on the surface of the film. The "evanescent wave" is that portion of the light which can interact with matter beyond the surface of the waveguide.

Referring to Fig. 4A, the waveguide **24** is made out of a light-conducting material which is sufficiently free from light-absorbing materials at the laser wavelength to avoid undue interference with the laser light passing through it. In a preferred embodiment of this invention, the waveguide **24** is planar. The refractive index of the waveguide material is greater than its surrounding medium, air, so that a laser beam **42** passed into the waveguide at an angle greater than the critical angle **26** is contained within the waveguide **24** and the evanescent waves **30** are present at the surfaces of the waveguide.

In qualitative terms, the larger the refractive index of the waveguide, (1) the better its ability to contain the laser beam; (2) the smaller the penetration of evanescent wave into the surrounding medium; and (3) the larger the launch angle to contain the beam within the waveguide. These factors are competitive. For

example, a larger index of refraction is desirable to contain the beam; however, a smaller index of refraction is desirable for greater penetration by the evanescent waves. The material selected as a waveguide is based upon a tradeoff off of these competing factors.

Typical materials for the waveguide are polystyrene, polypropylene, borosilicate (glass) and polycarbonate. Polystyrene is a preferred material due to its compatibility with long wavelength light in the near infrared range of the spectrum and its established efficacy and common use as an immunochemical substrate. Polystyrene waveguide material is commercially available from Polyfiltronics, Inc. 100 Weymouth Street, Rockland, Maryland 02370 (617) 878-1133.

Referring to Fig. 4A, thinner waveguides are more desirable than thicker waveguides. This is because thinner waveguides result in more bouncing of the laser beam off the first and second surfaces of the waveguide. This is known as a "higher mode of propagation" in the waveguide. A higher mode of propagation results in a better generation of evanescent waves **30** radiating from the full surface of the waveguide with a minimization to elimination of dark spots. The waveguide can be as thick as about 100 mils. Ideally, the waveguide is between about 1 mil to about 10 mils thick.

Referring to Fig. 1, typically the waveguide **24** is either rectangular or square shaped in cross-section. For a rectangular waveguide, the length of waveguide is about 1 inch to about 4 inches with a preferred length of 3 inches. The width of the waveguide is from between about 0.1 inches to about 1 inch with a preferred width of 0.25 inches. Longer waveguides can be used; however, as the length of the waveguide increases, there is a dropoff in laser beam intensity and a problem with the waveguide sagging. Some sag in the waveguide adversely affects the sensitivity or effectiveness of the assay. As technology for applying probes (described below) in discrete areas improves, the size of the waveguide can be reduced to that of a typical U.S. postage stamp and even smaller.

Optionally, channels are included in the waveguide to cordon the waveguide off into areas. This is done by either (i) a chemical or vapor deposition of a light-blocking material; (ii) printing on the waveguide; or (iii) use of a hydrophobic agent. The advantage of channels is to confine liquid samples to desired areas of the waveguide, or confine light to desired areas. This is useful in the simultaneous assay of multiple samples, as well as multiple target analytes.

Probes -- Referring to Fig. 1, the probes **20** include a specific binding partner, and can consist essentially of a specific binding partner bound to the waveguide. A specific binding partner is a first molecule which forms a complex or conjugate with a second molecule or substance referred to as the target analyte. The specific binding partner has a site which has a high avidity and affinity for the target analyte. High avidity means that the site specifically binds the target analyte to the exclusion of other substances, and high affinity means a strong association to the target analyte. The specific binding partner ("sbp") can be naturally occurring or artificially formed.

Many of the compounds conventionally used in diagnostic procedures as specific binding partners can be used for the probes **20**. Exemplary specific binding partners include:

(1) Single stranded polynucleotides of DNA ("deoxyribonucleic acids") and RNA ("ribonucleic acids") -- The sbp's have a length between about 5 bases to about 25 bases with a preferred length of about 12 bases. These sbp's hybridize to a single stranded DNA or RNA in the sample;

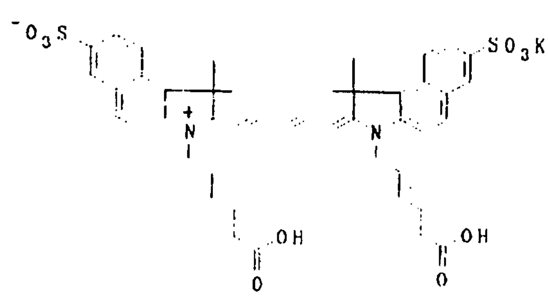
(2) Antibodies and fragments of antibodies -- The antibodies are of any of the various classes or subclasses of immunoglobulin, *e.g.*, IgG, IgA, IgM, IgD and IgE, and of either human or animal origin, such as sheep, rabbits, goats and mice. In addition to intact antibodies, antigen binding fragments are usable; *e.g.*, Fab Fab' and Fab'_2. The antibodies or fragment can be

produced by hybridoma technology, cloning and expression or display on a bacteriophage;

(3) Enzymes, receptors and other proteins having a binding site specific to a selected molecule.Ê

Light-responsive, light emitting and light-modulating compounds -- The term "light-responsive compound" refers to two types of molecules. First, it refers to molecules which are excited by evanescent waves so as to emit light; *i.e.*, light emitting compounds. Second, it refers to molecules which do not necessarily emit light themselves; rather, they enhance, quench or shift the wavelength of the excitation and light emission of another molecule. These light-responsive compounds are called "light-modulating compounds". The effect of a light-modulating compound is to emit, quench, enhance or shift the wavelength of light.

Suitable molecules which are excited by evanescent waves to emit light are fluorophores; namely, phthallocyanine dyes, LaJolla Blue (Si phthallocyanine with PEG axial ligands), fluorescein isothiocyanate ("FITC"), rhodamine isothiocyanate; 2, 4-dinitrofluorobenzene, phenylisothiocyanate, dansyl chloride, substituted rhodamine isothiocyanates ("XRITC"), tetraethyl rhodamine isothiocyanate ("TRITC"), cadaverine ("TRAP") and phycobiliproteins (*e.g.*, allophycocyanin, HDITCP (1,1', 3.3.3', 3' hexamethyl-4,4', 5.5' dibenzo-2,2' indotricar bocyanine percholoate), and phycoerythrin) fluorophores discussed in U.S. Patent No. 4,877,965, which is incorporated herein by reference, and the molecule illustrated herein below which has a carboxy acid tail for conjugation and is commonly known as DBCY5.



Suitable molecules which are excited by evanescent waves to emit light that have a large avidity and are specific to double stranded nucleic acids are ethidium bromide, acridine orange (C.I. No. 46005), quinacrine, diethidium bromide, diacridine orange and various heterodimers of the foregoing. U.S. Patent No. 5,268,486 to Waggoner discloses an arylsulfonate cyanine dye which bonds to DNA/RNA, and is incorporated herein by reference.

Suitable light-modulating compounds are cyclodextrins, H_2O_2, and other peroxide derivatives.

The following table presents light-modulating compounds useful in conjunction with various light-emitting compounds and the effect (associated property charge) of the light modulating compound.

Light-Emitting Compound	Light-Modulating Compound	Effect		
		E	Q	WLS
fluorescein	cyclodextrin			
fluorescein	rhodamine			
DBCY5	peroxide			

Wherein "E" stands for "Enhance," "Q" stands for "Quench," and "WLS stands for "Wavelength Shift.

Attaching probes to the waveguide -- Referring to Fig. 2, the configuration of the discrete areas **22** on the waveguide surface can be an array of parallel strips. Referring to Fig. 1, a preferred configuration of discrete areas on the waveguide surface is a matrix of rows and columns of spots. The shape of the discrete areas **22** can be any shape which allows for detection by a detector; *e.g.*, squares, triangles or stars. A preferred shape is spots (approximately circular) and strips (approximately rectangular). Spots are a more preferred shape to facilitate maximum use of the surface area of the waveguide.

The discrete areas **22** can be as small in size as the limit of the detection of the analysis.

Typically, the limits of detection require a discrete area size of at least about 20_^{2} with 1:1 imaging optics. The probes are applied in discrete areas on at least one surface of the waveguide. Referring to Fig. 3, probes **20** and **20'** can be applied to both surfaces of the waveguide.

U.S. Patent No. 5,429,807 to Mattson et al., which has been assigned to the assignee of the present patent, Beckman Instruments, Inc. (Fullerton, Calif.), and is herein incorporated by reference, discloses a method suitable for applying probes in discrete areas onto the waveguide. In particular, this patent discloses an automated method for performing macromolecule synthesis on a waveguide surface whereby a two-dimensional array of biopolymers in discrete areas are obtained on the surface.

Another method to apply probes in discrete areas on the waveguide is to use an ink jet printer. Typically, a commercial ink jet printer has about four jets which can each be loaded with a different probes. An ink jet printer intended for microfabrication of custom units typically has six jets which can be loaded with different probes. These commercial or microfabrication dedicated ink jet printers can be modified to provide for more "jets" so that a greater number of different probes can be applied in a single pass of the waveguide through the printer. The waveguide can be run through multiple ink jet printers wherein each ink jet printer applies probes. In the alternative, a stack of waveguide sheets are run through a single ink jet printer to apply probes. The jets are then cleaned and loaded with different probes and the process is repeated as many times as necessary.

For example, a band pass filter can be used on the light from the laser to be certain that only light of a desired wavelength passes into the waveguide, i.e., only light of a wavelength to which the dye is responsive. Similarly, an emission band filter can be provided between the waveguide and the detection apparatus, for filtering out the light of all wavelengths

other than those expected to be emitted by the chosen fluorophore.

The following U.S. patents, which are incorporated herein by reference, disclose methods and apparatus for bonding polynucleotides and/or polypeptides to polypropylene, polystyrene, glass and other waveguide materials: U.S. Patent No. 5,135,785 to Farnsworth; 4,065,412 to Dreyer; 4,704,256 to Hood; 4,603,114 to Hood; 3,652,761 to Weetal; 4,695,537 to Dorsett; 5,242,797 to Hirschfeld and 4,631,211 to Houghten. Methods for derivatization of polypropylene for bonding to polynucleotides are described in co-pending U.S. Patent application serial number 07/091,100, which has been assigned to the assignee of the present patent, Beckman Instruments, Inc. (Fullerton, Calif.), and is herein incorporated by reference.

Control probes can be used to test for error in running an assay. A control probe is responsive to control target analytes. One or more control probes are applied to the waveguide at discrete areas as previously described. The sample to be analyzed is spiked with control analytes. If the assay is functioning properly, there is a positive reading for the control analyte. Any failure to obtain a positive reading indicates a problem with the assay. The molecule used as a control is not a target analyte which is the subject of the analysis.

Water permeable overlayer -- Referring to Fig. 2, optionally, a water permeable overlayer **32** can be applied to the surfaces **23** and **25** of the waveguide **24**. Typically, the water permeable overlayer is a sol gel. Referring to Fig. 4B, the function of the water permeable overlayer **32** is to enhance the containment of the laser beam within the waveguide. In particular, there may be imperfections on the surfaces **23** and **25** of the waveguide (*e.g.*, scratches and cuts) through which light can refract out of the waveguide. The water permeable overlayer **32** catches this escaping light and reflects it within the waveguide-water permeable overlayer complex.

Typical sol gels are transparent oxide glasses which transmit light and are permeable to water. Sol

gels can be prepared by hydrolysis and polycondensation of alkoxides such as tetramethylorthosilicate. A typical method of preparation is discussed in *Science*, 255, 1113-1115 (1992) by Fink et al.

The refractive index of the sol gel can be modified by aging or mixing the composition with TiO_2 based materials.

The sol gel can be spin coated onto the waveguide. The spin coating can take place before or after contacting the waveguide with sample. Where the sol gel is spin coated on first, then the sample is applied on top of the sol gel and it permeates through the sol gel to the probes.

Multiple layers of probe on waveguide -- Referring to Fig.Ê3, an embodiment of the invention is to apply a first set of probes **22** to the first surface **23** and second set of probes **22'** to the second surface **25** of the waveguide **24**. Referring to Fig. 5, when a laser beam **42** is passed into the waveguide **24**, evanescent waves **30** and **30'** radiate out of both the first surface **23** and second surface **25** of the waveguide and excite probe-sample complexes on both the first surface **23** and second surface **25** of the waveguide **24**.

In another embodiment, probes are embedded in a three-dimensional array in a cube. The cube is a "layer cake" of waveguide with sol gel interspersed. In this case, the refractive index of the sol gel is made higher than that of the plastic so the sol gel becomes the waveguide.

Bonding light-responsive compound to sample or probe -- Methods for bonding a light-responsive compound to target analytes in a sample and/or to a specific binding partner at a probe are well known to those of ordinary skill in the art, and include methods such as those described in L.M. Smith et al., Nucleic Acids Research, Vol. 213, p. 2399 (1985); B.S. Packard et al., Biochemistry Biophysics ACTA, Vol. 769, p. 2010208 (1984); P.K. Bhattacharyya et al., Biochemistry Biophysics Research Communities, Vol. 101, p. 273-280 (1981); H. Haigler et al., Proceedings Of The National

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63:321A-327A, 1991; A. Riggin et al., Anal. Chem., 63:468-474, 1991; E. Sakal et al., Biochem., 30:8899-8904, 1991; R. Shimazawa et al., Biochem. Biophys. Res. Commun., 179:259-265, 1991; Y. Uchimura et al., Gene, 108:103-108, 1991; W. Volker et al., J. Histochem. Cytochem., 39:1385-1394, 1991; J. G. Westmur, CRC Crit. Rev. Biochem. Mol. Biol., 26:227-259, 1991; Yamada et al., Anal. Biochem., 199:132-136, 1991; Oser et al., Anal. Biochem., 191:295-302, 1990; and U.S. Patent No. 5,268,486 to Waggoner which has already been incorporated by reference. A preferred technique to bond a fluorophore to a target analyte in the sample and/or probe is to put a long chain carboxylic acid tail on the fluorophore. The fluorophore can then be bonded to amino or other nucleophilic groups using a coupling agent such as carbodiimide.

The sensitivity and versatility of the assay can be increased by using multiple light-responsive compounds where (1) certain probes have one light-responsive compound associated with them and other probes have another light-responsive compound associated with them and/or (2) different light-responsive compounds are bonded to different samples. The term "light-responsive compounds" refers to both "light-emitting compounds" and "light-modulating compounds". Associating different light-responsive compounds with different probes facilitates a detector to discriminate between probes at different discrete areas. This is achieved, for example, through the different light-responsive compounds emitting light at different wavelengths. This improves the versatility and sensitivity of the assay in that a light-responsive compound best suited for interacting with a particular target analyte in a sample can be associated with the probe for that target analyte. Another light-responsive compound best suited for interacting with a second target analyte in the sample can be associated with the probe for the second target analyte.

Associating different light-responsive compounds with different samples allows for analyzing more than one sample at a time. This is achieved, for

example, through the use of light-responsive compounds that emit light at wavelengths sufficiently different that a detector (discussed below) can discriminate between the wavelengths.

Laser light -- Referring to Fig.Ê5, a laser light source **38** can be a near infrared ("NIR") laser, a visible light laser or ultraviolet ("UV") laser. Long wavelength light in the near infrared range is preferred in that long light reduces background noise and facilitates the use of charge coupled detectors ("CCD"). In wet samples (described below), the wavelength preferred is no longer than about 1000 nanometers in that longer wavelengths result in interference caused by the laser light interacting with water in the wet sample and the Si of a CCD being non-responsive. In dry samples, because only the Si response is of concern, the preferred wavelength is from about 600 to about 960 nanometers. Short wavelengths in the ultraviolet range of the spectrum can also be used; but, ultraviolet light is not compatible to transmission through a polystyrene waveguide. The power output of the laser is from about 1 milliwatt to about 100 milliwatts. A diode laser (*e.g.*, gallium arsenide) is a preferred laser light source. This is because diode lasers are commercially available, inexpensive and have suitable power.

Commercially available diode lasers that are suitable for use in this invention are gallium arsenide laser which emit laser light at 650, 660 or 670 nanometers sold by Toshiba (Tokyo, Japan) as Model Numbers TOLD9321, TOLD9412, TOLD 9520; 750, 790 and 810 nanometer lasers sold by Sharp Corp. (Osaka, Japan) as Models LTU16, LTU24, LTU31,; and a laser with built-in optics sold by Lasiris, Inc. (St. Laurent, Quebec, Canada) as Model No. SNF 501-H.

The laser light source **38** emits a pencil-shaped beam **40** which needs to be reshaped into the slit-shaped light beam **42**. An optical device **44** that is non-Gaussian is preferred for reasons of its simplicity. Such an optical device is disclosed in U.S. Patent No. 4,826,299 and Canadian Patent, 1,276,829 which are incorporated

herein by reference. The device utilizes prisms to achieve a "line detector" function. Such a non-gaussian optical device is commercially available from Lasiris, Inc. (St. Laurent, Quebec, Canada), Model No. SNF-501-H and SLH-501-H.

Reshaping can also be accomplished using optics **44** that are gaussian, in particular, cylindrical optics, edge detector optics or spherical optics. Cylindrical optics are preferred. Referring to Figs. 5 andÊ6, the cylindrical optics comprise a first planoconvex cylindrical lens **46** and an optional second planoconvex cylindrical lens **48** which are substantially identical. The planoconvex cylinder lens have a planar face **50** and **50'**. Diametrically opposing each planar face is a convex face **52** and **52'**. The lenses are made out of quartz or borosilicate glass. The dimensions of the lens are between about to about 0.2" (l) x 0.1" (w) x 0.2" (d) to about 1" (l) x 0.5" (w) x 2" (d), with a preferred dimension of 0.5 inch (l) x 0.1 inch (w) x 0.2 inch (d). The focal length of the planoconvex cylinder lens is between about 3 mm. to about 20 mm, with a preferred focal length between 5 mm and 6 mm. The lens is commercially available from Melles Griot Corp., Irvine, California, Part #01 LCP 000 or 01 LCP 124.

A single planoconvex cylindrical lens is preferred. Alternatively, a system of two planoconvex cylindrical lenses is used. The two planoconvex cylindrical lenses **46** and **48** are positioned in a front-to-front configuration. A filter can be placed between the lenses.

The intensity of the emission of light from a laser diode follows a gaussian distribution. Despite this gaussian distribution, there usually is substantially equal illumination over the entirety of the waveguide. When the analytical procedure is taken to the limits of its sensitivity for detection, it may be necessary to correct for uneven illuminations. One of ordinary skill in the art is familiar with mathematical formulas to compensate for the gaussian distribution of laser light intensity. In the alternative to

mathematical correction, a light-emitting compound having an actual known emission is applied to the waveguide. This compound serves as an internal standard upon which to make corrections based upon the detected emission and actual known emission.

There can be a proximal prism **58** positioned at the end portion **56** of the waveguide **24** where light enters the waveguide. The purpose of this proximal prism **58** is to adjust the launch angle **26** of the slit beam into the waveguide so that it is less than the critical angle. Preferably, the prism adjusts the launch angle to provide the best signal-to-background ratio. The more desirable launch angles are from about 1 degree to about 39 degrees with 30 degrees being the most preferred.

Continuing to refer to Fig. 5, there optionally can be a coupling prism **60** at the distal portion **62** of the waveguide **24**; *i.e.*, the end of the substrate opposite the end where laser light exits the waveguide **24**. The purpose of this coupling prism **60** is to bleed out the laser light **42** and evanescent waves **30** from the waveguide **24**. In a configuration having coupling prisms **58** and **60** at both ends of the waveguide **24**, the prisms serve the additional purpose of being a stage for holding the waveguide **24**.

The coupling prisms are triangular blocks. Typically, the coupling prisms have dimensions from about 1 mm (l) x 1 mm (w) x 1 mm (d) to about 3 cm (l) x 3 cm (w) x 3 cm (d). The coupling prisms are made out of quartz or borosilicate glass. The index of refraction ranges from about 1.4 to about 1.6. A suitable prism known as a hemispherical prism is commercially available from Harrick Scientific, Ossining, New York.

Referring to Fig. 5, the interfaces between the prism **58** and **60** and the portions of the substrates **56** and **62** need to be substantially free from air bubbles. This is because air bubbles cause diffraction of the laser light resulting in background noise and a loss of laser intensity. One way to achieve a bubble free interface is to apply a coupling fluid to the interface. The coupling fluid has the same index of refraction as the prism.

Coupling fluid can be commercially purchased from Cargille Laboratories under the name REFRACTIVE INDEX MATCHING FLUID. The use of coupling fluid has the additional benefit of enhancing the lateral field uniformity. Another way to achieve a bubble free interface is to apply pressure to the prism to urge it against the waveguide so as to force out any air bubbles. This can be achieved through a clamping mechanism.

Detection device, imaging optics and confocal lens -- Referring to Fig.Ê5, a detector **64** used to detect emitted light can be a charge transfer device ("CTD"). A CTD has a two-dimensional multi-column layout, *i.e.*, pixels. When struck by light, each column (pixel) builds up charge and products an output signal. The light emitted at a discrete area on the waveguide will be focuses onto the active detecting surface of the CTD and will illuminate one or more pixels depending upon the area emitting and the magnification and focus of the collection optics. The charge accumulated on all illuminated pixels is added together into an output signal. This addition of cumulative charges is known as "binning." There are two principal types of CTDs; namely, a charge coupled device ("CCD") and a charge injector device ("CID"). CCDs are commercially available from Photometrics Ltd., Tucson, Arizona, and Princeton Instruments, Princeton, New Jersey.Ê

The detector **64** can be equipped with imaging optics, including a confocal lens **66**. The detector can be equipped with filters to block out light other than that at a selected wavelength. Such filters are used when the assay employs different light-responsive compounds that emit light at different wavelengths.

Target analytes -- An exemplary list of target analytes which can be tested for are: (1) hormones, including, but not limited to, insulin, follicle stimulating hormone, progesterone, estrone, testosterone, adrenalin (epinephrine) and noradrelin (norepinephrine); (2) illegal drugs, including, but not limited to, amphetamines, methaphetamines, mescaline, lysergic acid diethylamide (LSD), morphine and N-ethyl-3-

piperidylbenzilate; (3) immune factors, including, but not limited to, interferon, lucotrienes and macrophage coating stimulating factor; (4) cancer related molecules including, but not limited to, myelomas, neoembryonic antigens, epidermal like growth factor, insulin like growth factor and tumor narcosis factor; (5) antibodies to viruses and diseases, including, but not limited to, antibodies to hepatitis, AIDS (acquired immune deficiency disease), polio, measles, diphtheria and yellow fever; (6) toxins and poisons, including, but not limited to, arsenic, strychnine and bacterial endotoxins; and (7) miscellaneous blood components and proteins, including, but not limited to, immunoglobulins, complement proteins, low density lipoproteins, high density lip-proteins, cholesterol and serum albumin.

Methodology -- To run an assay, the sample is optionally pre-reacted with one or more light-responsive compounds. The light-responsive compound can be either a light-emitting compound or a light-modulating compound. Where the sample is not pre-reacted with a light-emitting compound, the probes on the waveguide include a light-emitting compound or a developer is used. Where a light-emitting compound is bonded to sample, it can be desirable to include a light-modulating compound with the probes on the waveguide.

The next step is to apply sample to the waveguide. This can be done by dipping, pouring, brushing or spraying. The waveguide is subjected to appropriate conditions to facilitate the formation of a complex between probe and any target analyte in the sample. This can require incubation at an elevated temperature, increasing or decreasing pH or salt concentration. For example, if the probe is an antibody, it can be necessary to incubate at an elevated temperature. If the probe is an oligonucleotide, it can be necessary to apply a wash solution to reduce salt concentration. The necessary reaction conditions and times are apparent to those of ordinary skill in the art based upon the particular target analyte and specific binding partner.

If a developer (*e.g.*, a monoclonal antibody or target analyte analog with a light-responsive compound attached to it) is used, it is applied to the waveguide in the same manner as sample, simultaneously or sequentially, depending on the assay type. The waveguide is then subjected to appropriate conditions to facilitate the formation of complex between the developer and target analyte-probe as described above with respect to sample-probe complex formation.

There is no need to dry the waveguide. That is, the analysis can be conducted "wet." Being able to conduct the analysis wet is one of the advantages of this version of the invention in that it saves the time and money of a drying step. Further, not having a drying step simplifies automated processing equipment. When running the analysis wet, the laser light source emits light between about 600 nanometers to about 960 nanometers. Light of a wavelength greater than 960 nanometers interacts with water in the wet sample to possibly lead to spurious results.

Optionally, the waveguide can be dried before passing a laser beam into it. It is desirable to dry the waveguide when the chosen light-responsive compound requires light of a wavelength greater than 960 nanometers or to improve sensitivity of the analysis. Optionally, a sol gel overcoat onto the waveguide, if one was not put on prior to applying sample. This is desirable where high sensitivity is required in that sol gel will reduce any diffuse reflectants.

A laser beam is passed into the waveguide and the emission of any light from each discrete area is detected.

Modified method of use for epi-illumination -- A version of this invention uses epi-illumination instead of passing a laser light into the substrate. This version of the invention follows the procedure previously discussed with the following modifications. Light-responsive compounds are selected which emit light at a wavelength different from that of the incident light. The waveguide is coated with a sol gel having an index of

refraction substantially equal to that of the waveguide. A light is passed into the waveguide at an angle about perpendicular to the plane of the waveguide. The detector is equipped with a filter to block out light at a wavelength equal to the wavelength of the incident light. The detection of light at a discrete area indicates the presence of the particular target analyte in the sample.

EXAMPLE 1

This example demonstrates an attempt to do quantitative analysis for Digoxin according to the present invention.

A waveguide was formed from polystyrene film, 4 mm thick. Printed onto it in spots was a monoclonal antibody for Digoxin. Multiple samples containing a predetermined concentration of free Digoxin were prepared, using Digoxin as a target analyte. The samples were spiked with a 10^{-9} molar concentration of biotinylated Digoxin, which functioned as an analog to compete with the free Digoxin for binding to the monoclonal antibody on the waveguide. The spiked samples were applied to the waveguide, and washed with water. After washing, a fluorescently labelled antibody to Digoxin was applied to the waveguide. The fluorescent dye used was DBCY5 synthesized with an NHS ester group for reaction with the amine group on the antibody. The dye labelled antibody bonded to the target analyte attached to the waveguide, but did not bond to the biotinylated Digoxin. The assay was conducted with a phosphate buffer to maintain a pH at about 7.6. After each sample was applied to the waveguide, the sample was provided with a 30-minute incubation period, and then blown dried.

Laser light from a gallium aluminum arsenide laser at about 670 nanometers was applied to the waveguide, and used for activating the dye.

Fig. 7 presents the response curve from the test. The curve shows this technique was good at qualitative analysis, but of limited success at quantitative analysis. Later experiments revealed that

for accurate quantitative analysis, it is preferred that the specific binding partner be covalently bonded to the waveguide.

EXAMPLE 2

This example demonstrates qualitative detection of a DNA oligonucleotide target analyte.

The waveguide used was made of polypropylene film, about 3 ml thick. A short sequence oligonucleotide complementary to the target analyte was covalently coupled to the waveguide using acyl fluoride coupling, and served as the probe. Both the target and the probe were about 10 to 15 nucleotides long. The target analyte was pretreated with CY5 dye, and also provided with an avidin linkage. The CY5 dye is the same as the DBCY5 dye shown above, without the two outermost benzene rings. The probe was provided with a biotin linkage. The waveguide had on one of its surfaces a layer of rows and columns of spots of the probe. The sample was applied to the waveguide, incubated, and dried. A laser provided light at 670 nanometers.

Fig. 8 presents the results. In Fig. 8 a lighted spot indicates attachment of the target oligonucleotide to the probe, while a dark spot means that no target oligonucleotide was in a sample.

The system of the present invention has significant advantages. It allows for the inexpensive and fast analysis of samples for multiple analytes using a straightforward and simple process. It can accurately detect target analytes. Moreover, the detector used in the system can be manufactured by conventional printing equipment. The method can be automated with non-complex equipment, it can be used for both qualitative and quantitative detection.

As a most preferred version of the present invention, as presently envisioned, the waveguide is made of polystyrene, where the specific binding partner is avidin attached to the polystyrene with a photocoupling technique such as that available from BSI Surface Microfinish Sciences of Eden Prairie, Minnesota, and sold

under the trademark "PHOTOLINK". A sample is treated with an antibody to the target analyte, the antibody having attached to it DBCY5 fluorophore. An analog to the target analyte is used, the analog comprising the target analyte bound to biotin. The laser is a gallium aluminum arsenide laser emitting light at about 670 nanometers. In this preferred method, the target analyte bound to the fluorophore attaches to the probe on the waveguide through an avidin-biotin linkage. The more target analyte present in the sample, the less analog binds, and the weaker the emitted signal.

Additional information regarding the present invention can be found in the attached report, entitled "NIR Fluorescence Imaging Detection of Ligand Binding on a Planar Evanescent Wave Sensor" which is incorporated herein by reference.

Although the present invention has been described in considerable detail with reference to certain preferred versions thereof, other versions are possible. For example, a band pass filter can be used on the light from the laser to be certain that only light of a desired wavelength passes into the waveguide, i.e., only light of a wavelength to which the dye is responsive. Similarly, an emission band filter can be provided between the waveguide and the detection apparatus, for filtering out the light of all wavelengths other than those expected to be emitted by the chosen fluorophore. Therefore, the spirit and scope of the appended claims should not be limited to the description of preferred versions contained herein.

What is claimed is:

1. A method for detecting a target analyte in a sample, the method comprising the steps of:

a) providing a detector comprising a waveguide having thereon a plurality of discrete probes, each probe including a specific binding partner for a selected analyte, at least one of the probes being a responsive probe that includes a specific binding partner for the target analyte;

b) applying the sample to the detector such that the target analyte binds to its specific binding partner;

c) passing laser light into the detector so that evanescent light radiates from the waveguide and impinges on the probes, wherein light, if any, emitted from a probe with target analyte bound thereto is different from the light, if any, emitted by the same probe without target analyte bound thereto; and

d) detecting emission of light from the probes.

2. The method of claim 1 wherein a probe without target analyte bound thereto emits substantially no light and a probe with target analyte bound thereto emits sufficient light to be detected during the step of detecting.

3. The method of claim 2 comprising the step of attaching to the target analyte, before or after the target analyte binds to its specific binding partner, a light-emitting compound, wherein the light-emitting compound emits light when the laser light is passed into the detector.

4. The method of claim 3 wherein the light-emitting compound is attached to the target analyte before the sample is applied to the detector.

5. The method of claim 1 wherein each probe

includes a light-emitting compound.

6. The method of claim 1 wherein the probes include a light-emitting compound, and the method comprises the step of attaching to the target analyte, before or after the target analyte binds to its specific binding partner, a light-modulating compound, wherein the light-modulating compound affects the light emitted by the light-emitting compound when the laser light is passed into the detector.

7. The method of claim 6 wherein the light-modulating compound is a quencher that prevents the light-emitting compound from emitting light.

8. The method of claim 6 wherein the light-modulating compound is attached to the target analyte before the sample is applied to the detector.

9. A method for detecting a target analyte in a sample, the method comprising the steps of:

a) providing a detector comprising a waveguide having thereon a plurality of discrete probes, each probe including a specific binding partner for a selected analyte, a plurality of the probes being a responsive probe that includes a specific binding partner for the target analyte;

b) exposing the probes to (i) the sample and (i) an analog of the target analyte, the analog being capable of binding to the same specific binding partner to which the target analyte can bind, so that the analog and the target analyte competitively bind to the responsive probes;

c) passing laser light into the detector so that evanescent light radiates from the waveguide and impinges on the probes, wherein light, if any, emitted from a probe with target analyte bound thereto is different from the light, if any, emitted by the same probe with the analog bound thereto; and

d) detecting emission of light from the probes.

10. The method of claim 9 wherein the analogs include a light-emitting compound so that only responsive probes with analog bound thereto emit light.

11. A method for analyzing multiple samples for the same target analyte, the method comprising the steps of:

a) providing a detector comprising a waveguide having thereon a plurality of discrete probes, at least some of the probes being a responsive probe that includes a specific binding partner for the target analyte;

b) treating each sample with a different light-emitting compound so that the light-emitting compounds attach to the target analyte in the samples to form conjugates;

c) applying the samples to the detector such that the conjugates bind to the responsive probes;

d) passing laser light into the detector so that evanescent light radiates from the waveguide and impinges on the responsive probes resulting in light being emitted by the light-emitting compounds, wherein the different light-emitting compounds emit different light; and

e) detecting emission of light from the probes.

12. The method of claim 11 wherein the different light-emitting compounds emit light that differs in the wavelength, intensity or color.

13. A method for detecting multiple target analytes in a sample, the method comprising the steps of:

a) providing a detector comprising a waveguide having thereon a plurality of different discrete probes, each probe including a specific binding partner for a selected analyte, at least some of the probes being capable of binding to a corresponding target analyte;

b) applying the sample to the detector such that the target analytes bind to their corresponding specific binding partner;

c) passing laser light into the detector so that evanescent light radiates from the waveguide and impinges on the probes, wherein light, if any, emitted from a probe with target analyte bound thereto is different from the light, if any, emitted by the same probe without target analyte bound thereto; and

d) detecting emission of light from the probes.

14. The method of claim 13 wherein a probe without target analyte bound thereto emits substantially no light and a probe with target analyte bound thereto emits sufficient light to be detected during the step of detecting.

15. The method of claim 13 comprising the step of treating the target analytes with a plurality of different light-emitting compounds, each light emitting compound attaching to a corresponding target analyte, wherein the light-emitting compounds emit light when the laser light is passed into the detector, the different light-emitting compounds emitting different light.

16. The method of claim 15 wherein the light-emitting compounds are attached to the target analyte before the sample is applied to the detector.

17. A detector for detecting a target analyte comprising:

(a) a waveguide having first and second opposing surfaces and an index of refraction greater than its surrounding medium such that laser light passed into the waveguide at an angle of incidence greater than a critical angle results in evanescent light; and

(b) a plurality of discrete probes on the waveguide, each probe including a specific binding partner for a selected analyte, at least one of the

probes being a responsive probe that includes a specific binding partner for the target analyte.

18. The detector of claim 17 wherein the waveguide is substantially planar.

19. The detector of claim 17 wherein probes include a light-emitting compound.

20. The detector of claim 19 wherein the light-emitting compound is a fluorophore.

21. The detector of claim 17 wherein a light-modulating compound is included with the probe.

22. The detector of claim 21 wherein the light-modulating compound is a quencher.

23. The detector of claim 21 wherein the light-modulating compound is an enhancer.

24. The detector of claim 21 wherein the light-modulating compound is a wavelength shifter.

25. The detector of claim 17 wherein different light-responsive compounds are included with different probes.

26. The detector of claim 17 wherein the probe areas are in a two-dimensional array.

27. The detector of claim 26 wherein the shape of the probes is spots.

28. The detector of claim 17 wherein the shape of the probes is stripes.

29. The detector of claim 17 wherein there are a plurality of probes on the first and second surfaces of the waveguide.

30. The detector of claim 17 further comprising a water permeable overlayer on at least one of the surfaces, the overlayer having a refractive index less that the refractive index of the waveguide.

31. The detector of claim 30 wherein the water permeable overlayer is a sol gel.

32. A detector for chemical analysis comprised of:

(a) a waveguide having opposing first and second substantially planar surfaces and an index or refraction greater than its surrounding medium such that laser light passed into the waveguide at an angle of incidence greater than a critical angle results in evanescent light;

(b) a plurality of probes of known recognition to selected target analytes in a two-dimensional array of discrete areas on at least one of the substantially planar surfaces; and

(c) a light-emitting compound included with the probes.

33. The detector of claim 32 wherein the light-emitting compound is a fluorophore.

34. The detector of claim 33 wherein there is a first probe having a first fluorophore which emits light at a first wavelength and a second probe having a second fluorophore which is different from the first fluorophore and which emits light at a second wavelength different from the first wavelength.

35. The detector claim 33 wherein there is a water permeable overlayer on at least one surface of the waveguide, the overlayer having a refractive index less than the refractive index of the waveguide.

36. The detector of claim 32 wherein the water permeable overlayer is a sol gel.

37. An apparatus for measuring the excitation of a light-emitting compound by evanescent waves comprised of:

(d) a laser light source emitting an approximately pencil-shaped beam;

(e) slit shaping optics positioned to receive the pencil-shaped laser light beam so as to transform the beam into a slit shaped light beam;

(f) a planar waveguide having a refractive index greater than its surrounding medium positioned to receive the slit shaped laser beam so that there is an angle of incidence greater than a critical angle which results in evanescent waves; the waveguide having the light-emitting compound therein and being excited by the evanescent waves; and

(g) a detection device positioned to measure light emitted from the light-emitting compound.

38. The apparatus of claim 37 including a proximal prism contacting the waveguide where it receives the slit shaped laser beam such that the slit shaped laser beam passes first through the prism and then into the waveguide, the prism serving to adjust the angle of incidence.

39. The apparatus of claim 37 including a distal prism contacting the waveguide at a distance away from where it receives the slit shaped laser beam, the distal prism being for bleeding the laser beam out of the waveguide.

40. The apparatus of claim 37 with the additional element of a confocal lens between the detector and the waveguide such that the detector can be focused at a particular depth in relationship to the waveguide.

41. The apparatus of claim 37 wherein the laser light source emits light between about 600 nanometers and about 960 nanometers.

42. The apparatus of claim 37 wherein there are different light-emitting compounds on the waveguide.

43. A method for analyzing a sample containing at least one target analyte using a light-responsive compound, the method comprising the steps of:

(a) providing a substrate having thereon a plurality of probes of known recognition to selected target analytes in discrete areas with an overlayer having an index of refraction substantially equal to or greater than the index of the refraction of the substrate, the overlayer being water permeable;

(b) applying the sample to the probes on the substrate such that the target analyte binds to corresponding probes;

(c) illuminating the substrate with epi-illumination; and

(d) detecting the emission of any light at the discrete areas.

44. The method of claim 43 wherein the overlayer is a sol gel.

45. A method for analyzing a sample containing at least one target analyte, the method comprising the steps of:

(a) providing a substrate having thereon a plurality of probes of known recognition to selected target analytes in discrete areas with a light-responsive compound included with the probes, and an overlayer having an index of refraction substantially equal to or greater than the index of refraction of the substrate, the overlayer being water permeable;

(b) providing an aqueous sample containing at least one target analyte;

(c) applying the sample to the probes on the substrate such that target analytes bind at corresponding probes;

(d) illuminating the substrate with epi-illumination; and

(e) detecting the emission at the discrete areas of any light of a wavelength different than that of the epi-illumination.

46. The method of claim 45 wherein the water permeable overlayer is a sol gel.

47. An article for chemical analysis comprised of:

(a) a substrate suitable for epi-illumination having first and second surfaces;

(b) a plurality of probes of known recognition to selected molecules in discrete areas on at least one of the surfaces of the substrate; and

(c) an overlayer on at least one of the surfaces of the substrate having thereon probes, the overlayer having substantially the same or greater index of refraction as the index of refraction of the substrate, the overlayer being permeable to water.

48. The article of claim 46 wherein the water permeable overlayer is a sol gel.

49. The article of claim 47 with the additional element of a light-responsive compound included with at least some of the probes.

50. The article of claim 49 wherein there are a plurality of probes in discrete areas on the first and second surfaces of the substrate.

51. An article for chemical analysis comprised of:

(a) a substrate having opposing first and second substantially planar surfaces and suitable for epi-illumination;

(b) a plurality of probes of known recognition to selected molecules in discrete areas on at least one of the substantially planar surfaces of the substrate;

(c) a fluorophore included with the probes; and

(d) a water permeable overlayer overlaying the surfaces of the substrate having thereon probes, the overlay having substantially the same or greater index of refraction than the index of refraction of the substrate, the overlayer being permeable to water.

52. The article of claim 51 wherein the water permeable overlayer is a sol gel.

53. The article of claim 51 wherein there are a plurality of probes in discrete areas on the first and second substantially planar surfaces of the substrate.

54. The method of claim 1 comprising the additional step of drying the waveguide before passing laser light into it.

55. The method of claim 3 wherein the light-emitting compound is a fluorophore.

56. The method of claim 1 wherein the laser light has a wavelength of from about 600 to about 960 nanometers.

57. The method of claim 1 wherein the specific binding partners are covalently bonded to the waveguide.

58. The detector of claim 17 wherein the specific binding partners are covalently bonded to the waveguide.

59. The detector of claim 32 wherein the specific binding partners are covalently bonded to the waveguide.

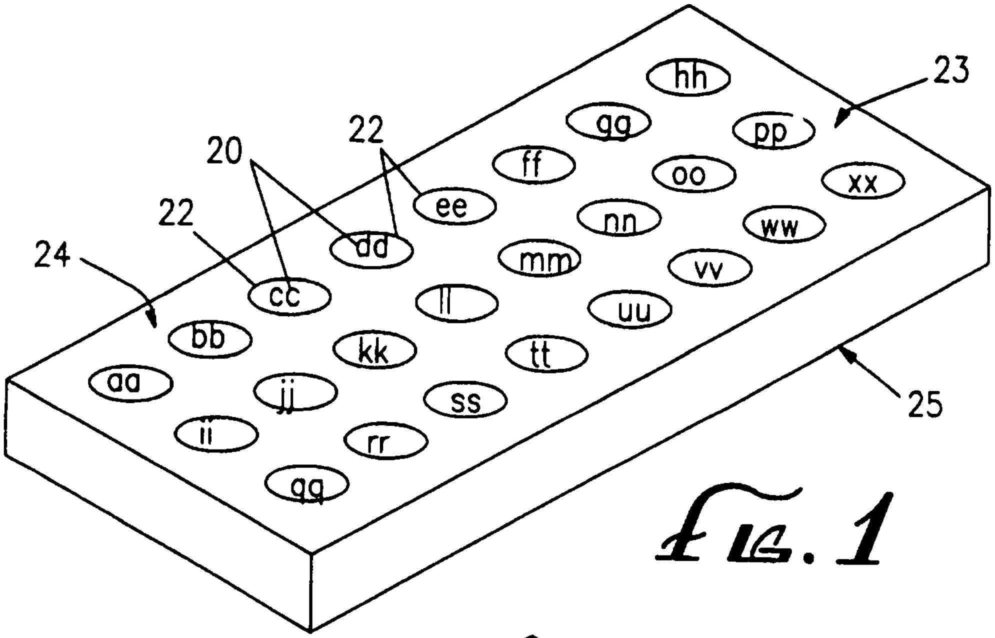
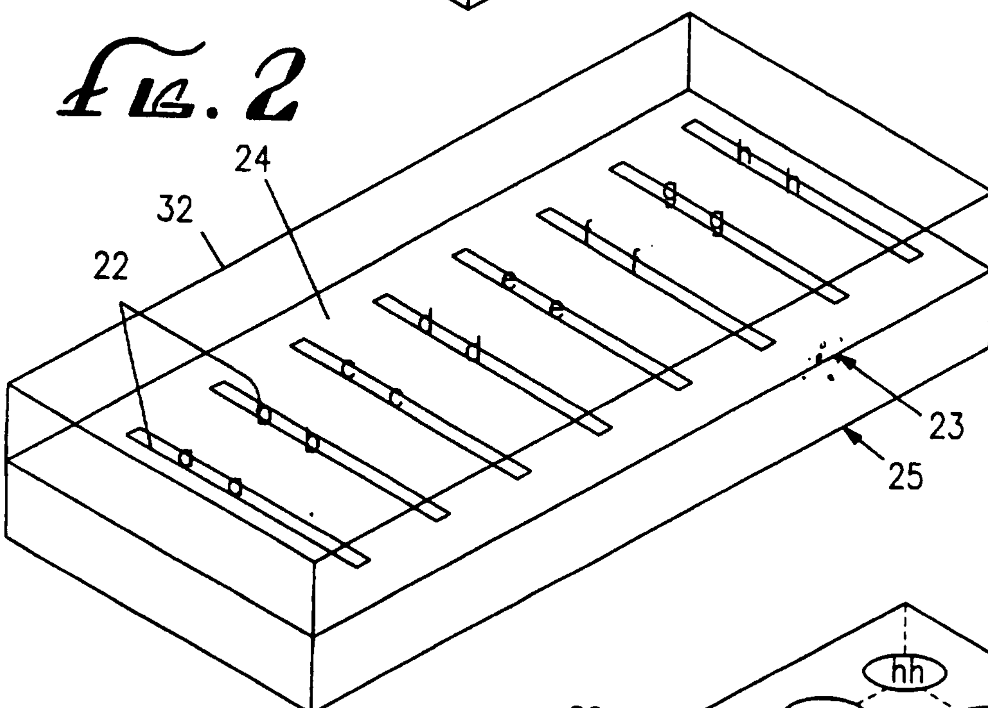


FIG. 1

FIG. 2



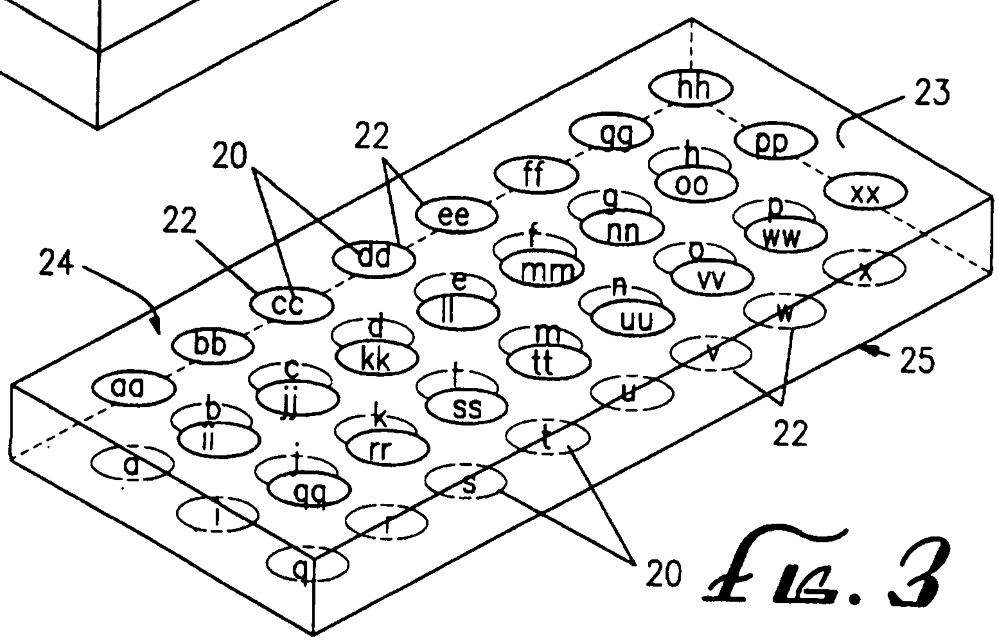


FIG. 3

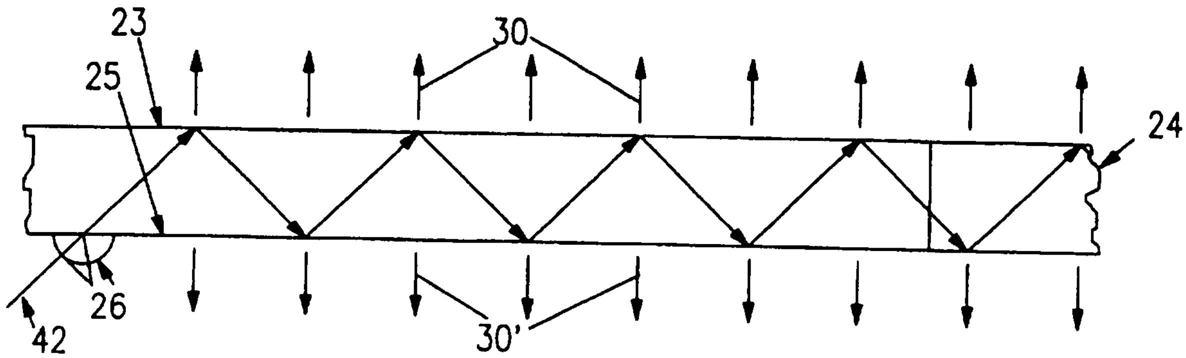


FIG. 4A

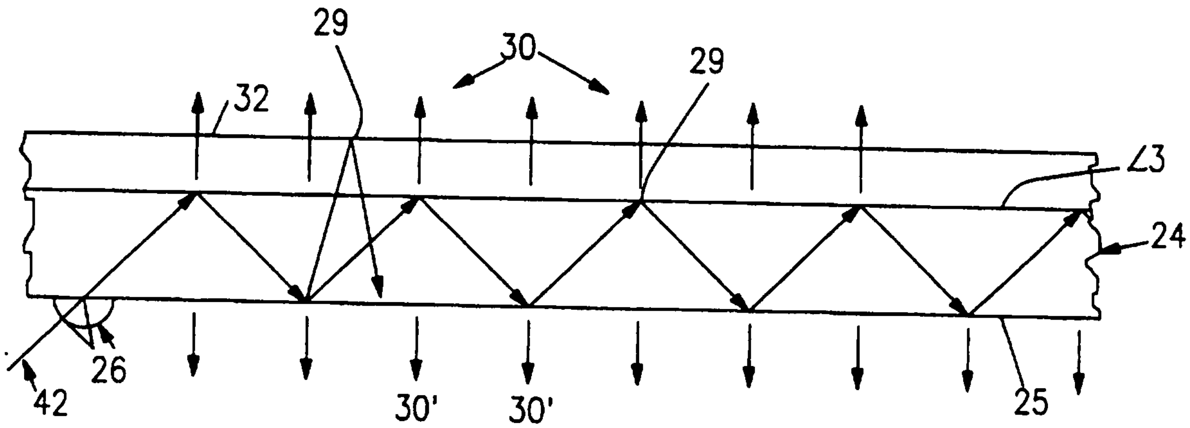


FIG. 4B

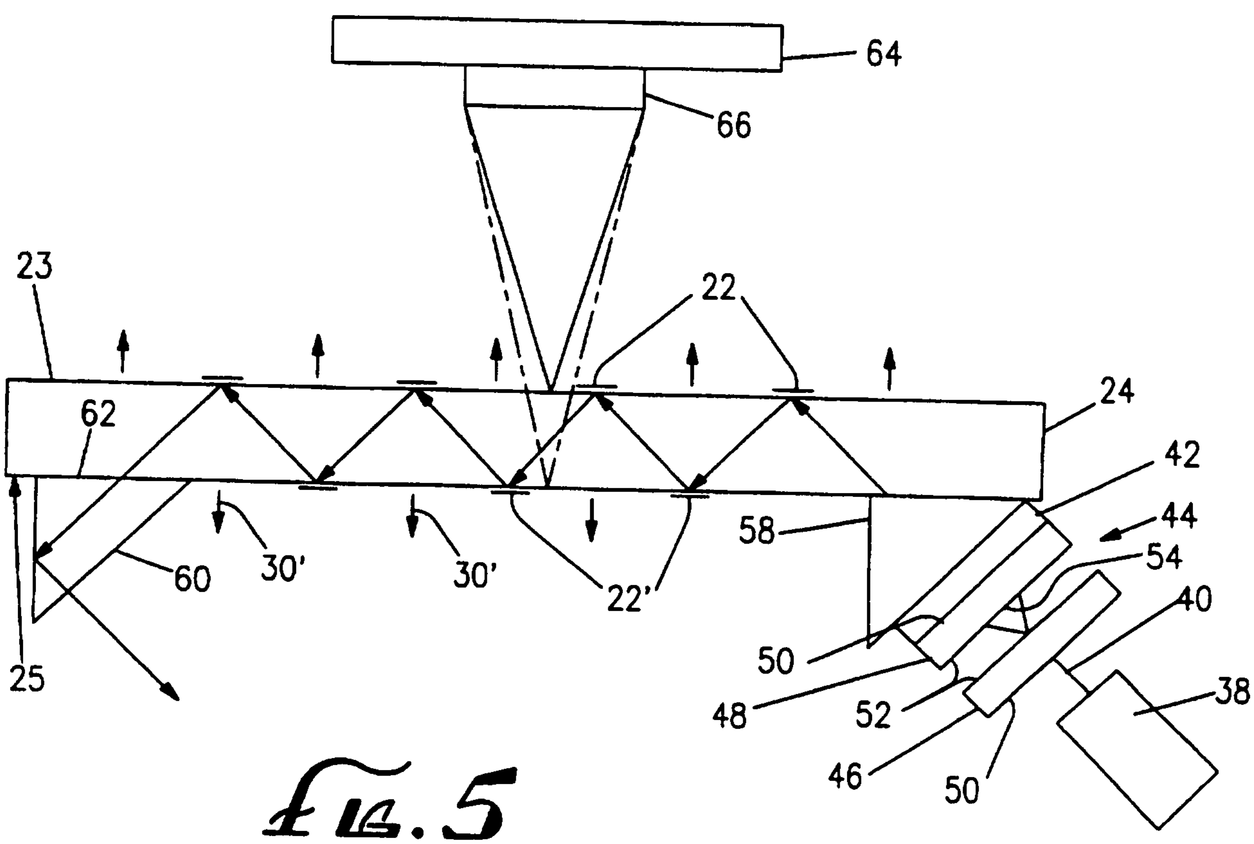


FIG. 5

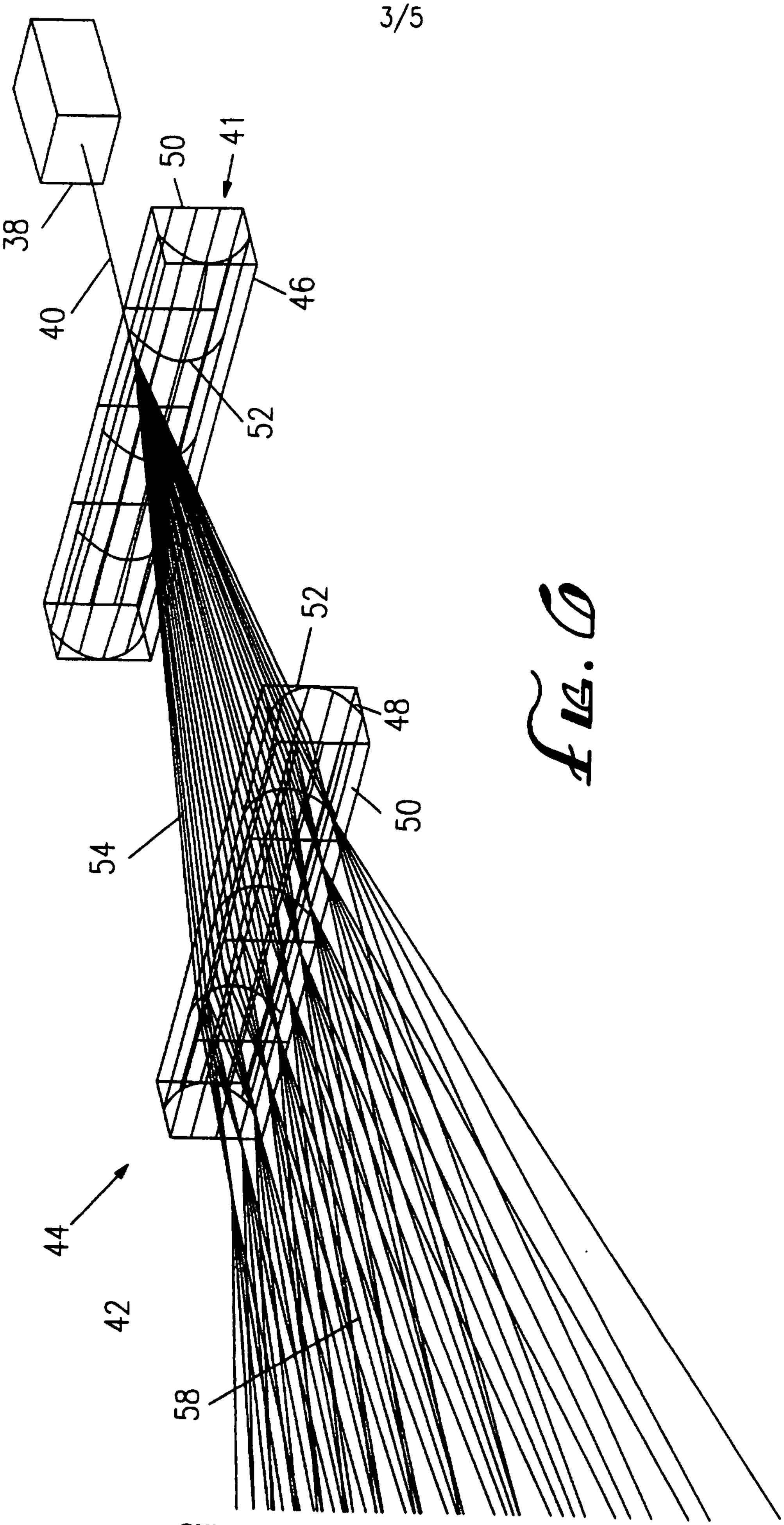
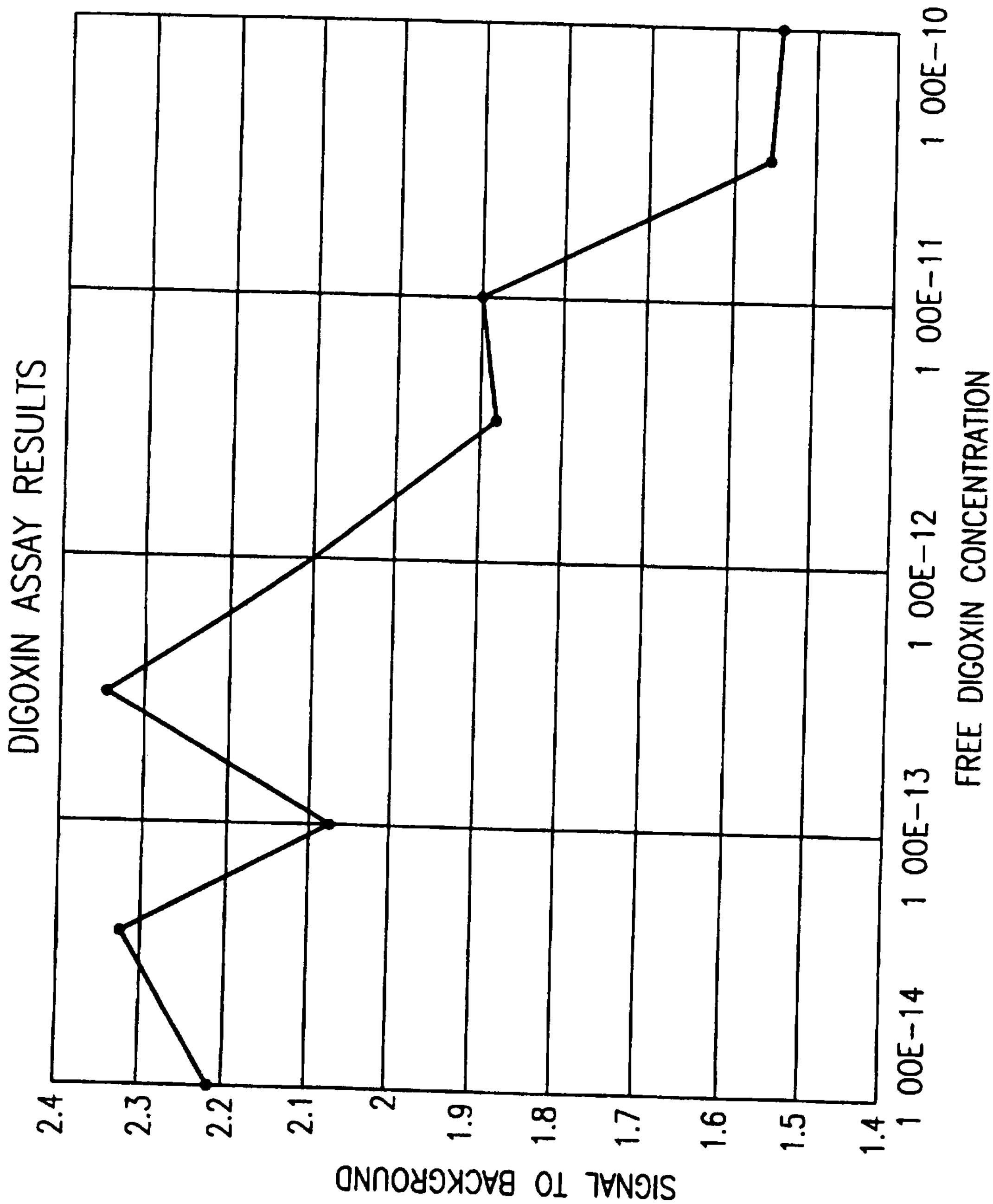


FIG. 6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1.4
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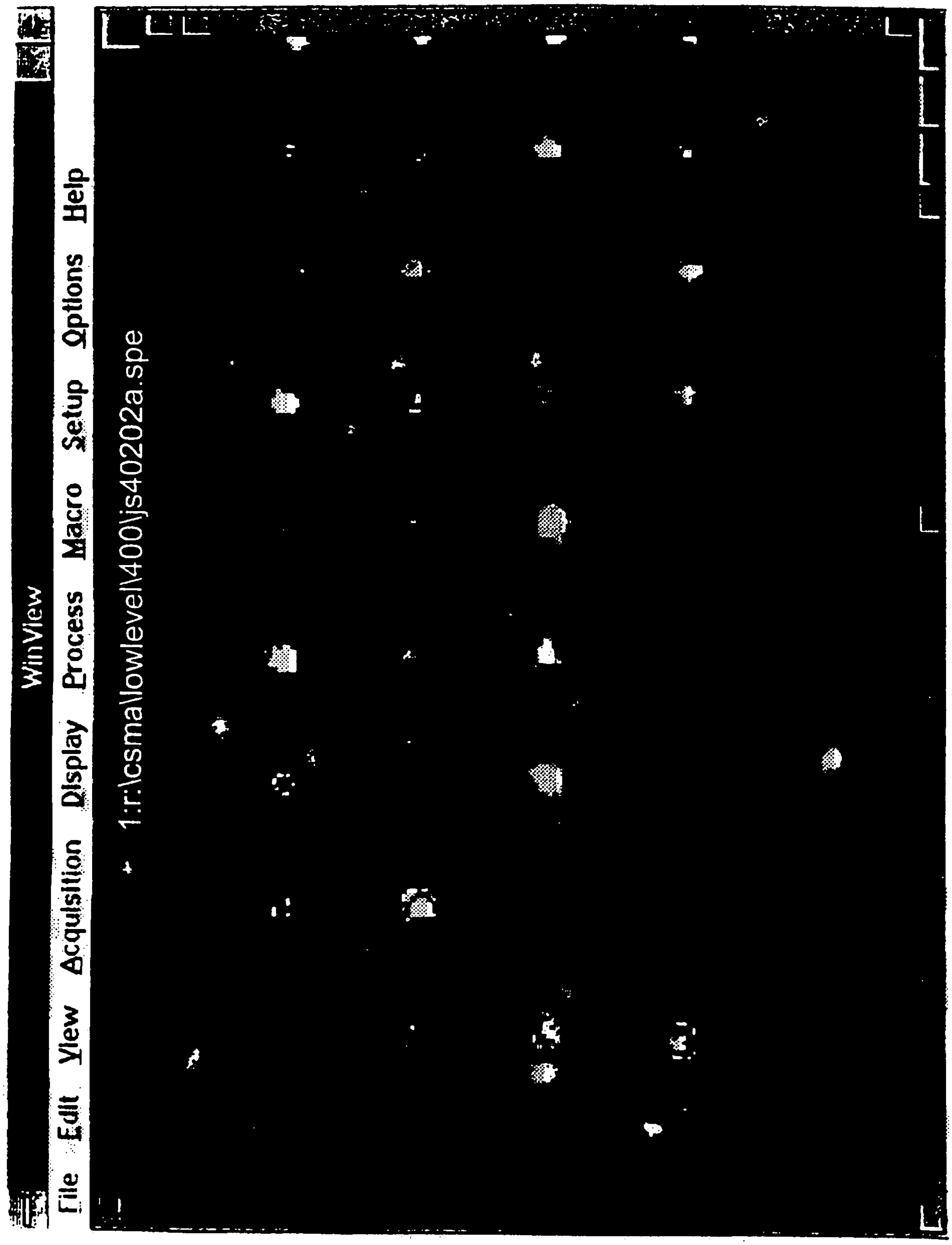
1 00E-13
1 00E-12
1 00E-11
1 00E-10
FREE DIGOXIN CONCENTRATION

FIG. 7

Fig. 8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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543 G01N21/55 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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1

Date of the actual completion of the international search	Date of mailing of the international search report
3 July 1997	16.07.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer Griffith, G

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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